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Sex Determination and the Restriction of Sex-linked Pigment Patterns to the *X* and *Y* Chromosomes in Populations of a Poeciliid Fish, *Xiphophorus maculatus*, from the Belize and Sibun Rivers of British Honduras[1]

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(Plates I-II; Text-figure 1)

X. maculatus is polymorphic for sex chromosomes and sex-linked pigment patterns. Females of natural populations may be of the genotype WY, WX, or XX and males XY or YY. Fish from the two rivers were tested for their sex-genotypes, because earlier but limited data had indicated that the X is absent from rivers in British Honduras. Of 8 males and 30 females tested, X chromosomes were only found in one male (XY) and female (XX). One WY female was fertilized by an XY male, before she was collected. Of the 29 females with a W, all but two exhibited one or more sex-linked pigment pattern controlled by at least three loci. The 27 females possessed a total of 41 factors. None was W-linked. In preserved collections from these rivers, the frequency of males with macromelanophore patterns was two to three times that of females. This difference is in good agreement with the hypothesis that in natural populations the W chromosome does not carry pigment factors. This is not true for the X chromosome. Since crossing over beween the W and Y has been observed in the laboratory, it must also occur in natural populations. In the absence of selection, crossing over should tend to equalize the frequency of marked W and Y chromosomes. A selective advantage is postulated for high coloration in males and a disadvantage in females. The significance of the W as a vehicle for strict maternal transmission of characters is discussed.

INTRODUCTION

THE SOUTHERN platyfish, *Xiphophorus maculatus*, has been the subject of many investigations, because of its unusual pigmentary and sex chromosome polymorphism. In *X. maculatus*, which ranges from southern Mexico near Veracruz to British Honduras, females may be of the sex genotypes *XX*, *WX*, or *WY* and males may be *XY* or *YY*. The sex chromosomes have not been identified cytologically, but an abundance of data concerned with sex ratios and the inheritance of sex-linked pigment patterns attest to the reality of the *W*, *X*, and *Y* chromosomes (Bellamy 1922, 1924, 1928; Bellamy and Queal 1951; Fraser and Gordon 1929; Gordon 1927, 1937, 1946, 1947, 1952; Kallman 1965).

The geographical distribution of the *W* and *X* chromosome has been the subject of some controversy and misunderstanding. Based upon experiments with platyfish obtained through commercial sources it was thought that sex determination in this species was of the *WY-YY* type (Bellamy 1922, 1924, 1928; Breider 1942; Gordon 1927, 1937; Kosswig 1938). In 1947 Gordon discovered the *X* chromosome in Mexican populations of platyfish and later stated that *X. maculatus* with an *XX-XY* mechanism inhabited rivers in Mexico and were geographically isolated from populations with a *WY-YY* system in British Honduras (Gordon 1954). This theory has been widely accepted in many review papers and monographs on sex deter-

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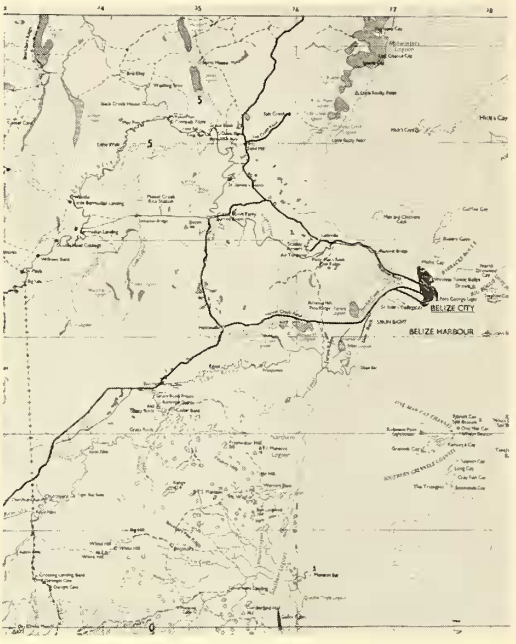
mination, but it is now outdated. More recent experiments with fish collected in several major drainages have shown that *W* and *X* chromosomes occur together in roughly 70 percent of the total known range of this species (Kallman 1965). The two female determining chromosomes, *W* and *X*, have been found in the Rio Grijalva, Rio de la Pasion (Rio Usumacinta system), Lake Peten, and Rio Hondo. Only in populations of the Rio Jamapa, Papaloapan, and Coatzacoalcos in the western part of the species range, has the *W* not been demonstrated; the *X* is not known from the New River and Belize River in British Honduras. Because relatively few fish were tested, the failure to demonstrate the *W* or *X* chromosomes from these rivers may be due to a sampling error. Kallman (1965) and Kallman and Atz (1967) have suggested that the sex chromosome mechanism of *X. maculatus* probably has arisen from a *XX-XY* type as is present today in *X. v. variatus, X. v. xiphidium, X. milleri,* and *X. p. nigrensis.* The sex chromosomes of the four species are homologous (Kallman and Atz 1967; Zander 1968). Some sort of selective advantage must have been and perhaps still is associated with the *W* chromosome, since it is widespread today.

To understand the evolution of the sex determining system of *X. maculatus,* it is important to establish whether the *X* is absent from any major river system. Such populations could have arisen as the result of the replacement of the *X* by the *W;* or they could have been founded by fish already possessing the *WY-YY* mechanism. This paper is concerned with sex determination of the platyfish populations inhabiting the Belize River and Sibun River of British Honduras.

Material and Methods

The three collecting stations can be found on Text-fig. 1. Fish from the Sibun River were collected at a locality called Freetown, in a small weedy pond on the left side of the dirt track that branches off the Western Highway about 2 km beyond Hattieville. Fish were caught by repeated sweeps with a 10 feet long, 4 feet wide (one quarter inch mesh) nylon seine along 20 meters of shore line. Because of dense shrubbery other parts of the bank were not accessible. The location, hereafter designated Freetown, was visited on Jan. 20 and 23, 1966. The data presented in Table 7 represent the combined count of the two collections. The ten females and two males which were brought alive to the Genetics Laboratory were given pedigree number 1899 (Table 1 and Table 2) and with the exception of male 1899-12 (Table 1) were collected during the second visit.



Text-fig. 1. Map of British Honduras showing the environs of Belize City. Collecting stations in the Belize River drainage are on the north side of the road leading to Bermudian Landing (above the "u" of Bermudian) and Gabourel Creek near Stanley airport. Collecting station for the Sibun River is at Freetown.

Fish from the Belize River were caught in a shallow, broad lagoon, located in a cow pasture on the right side of the dirt road running from the town of Boom towards the ferry crossing at Bermudian Landing. The exact location is "Mamre Farm," approximately 2 km east of the ferry. The data in Table 7 represent the combined count of two collections made on Jan. 21 and 24. The fish used for breeding purposes were collected on Jan. 24 and were assigned pedigree number 1900 (Table 1 and Table 3). This collecting station will be referred to as "Bermudian Landing."

The second collecting spot along the Belize River was Gabourel Creek, a ditch one to two meters deep that extends from the eastern limit of the main runway of the Belize airport (Stanley Field) towards the Belize River 1 km away. All fish were caught on Jan. 22, 1966, in close proximity to where the creek runs below the access road to Stanley Field; those kept alive for genetic experiments were given pedigree number 1901 (Table 1 and Table 4).

The sex genotypes of the males collected in the Sibun River and Belize River were identified by mating them to *XX* females of the Genetics Laboratory reference stocks (Kallman 1965). Males of the genotype *YY* sire all male broods. Males with the *XY* constitution give rise to offspring of both sexes in equal frequency; the paternal sex-linked pigment pattern, if present, is inherited by one sex only.

The sex chromosomes of the females from the two rivers were identified by either one of two methods. Some fish were mated to *XY* males of the reference stocks in which the *X* and *Y* chromosomes were differently marked. Females with the *WY* genotype give rise to a 1:1 sex ratio with both the *X*- and *Y*-linked paternal pigment patterns exhibited by one half of the offspring of both sexes. A 1:1 sex ratio is also obtained with *XX* females, but the *X*-linked pattern of the male parent is inherited by females and the *Y*-linked pattern by males only. *WX* females produce broods with a ratio of 3 ♀♀: 1 ♂♂: the *X*-linked paternal pattern is inherited by two-thirds of the female but none of the male offspring; the *Y*-linked pattern of the male parent is inherited by every male offspring but by only one third of the females.

Other females of the Sibun River and Belize River were mated to males known to be *YY*. Females with the genotype *XX* give rise to all male broods. Both *WY* and *WX* females give rise to a 1:1 sex ratio, but can be told apart by mating a male offspring of the F_1 generation to a *XX* female of the reference stocks. The male offspring of *WX* females are *XY* and sire broods that consist of both sexes; those of *WY* females are *YY* and give rise to all male progeny.

The identification of the sex genotypes of newly-collected females is greatly facilitated by the presence of sex-linked pigment patterns. Therefore most fish shipped to the Genetics Laboratory were marked. In this respect the breeding data reported in this paper represent a biased sample. In the field the fish of each seine haul were immediately examined by the author for pigment patterns. Fish were kept alive if they exhibited a pattern or combination of patterns net yet present in the collection. Usually not more than two fish with idential markings were selected from each location.

The following sex-linked pigment patterns, many of which are new for *X. maculatus* and which will be described in a forthcoming paper, were present in the fish from the Belize River and Sibun River.

Macromelanophore pattern:

N — Nigra: irregular black blotches along flank.

TABLE 1. SEX CHROMOSOME CONSTITUTION OF EIGHT MALE *Xiphophorus maculatus* FROM THE SIBUN RIVER AND BELIZE RIVER, BRITISH HONDURAS

Pedigree and genotype of parents			*Pedigree of offspring*	*Phenotype of offspring*				
	♀♀	♂♂		♀♀			♂♂	
Hp-2	X_+X_+	1899-11 $Y_+Y_{Ly}{}^1$	1919	none			15 Ly	22+
Hp-2	X_+X_+	1899-12 $Y_{Ly}Y_{Ir}$	1935	none			13 Ly	9 Ir
Hp-2	X_+X_+	1900-11 $Y_+Y_{Mr\delta d}$	2016	none			8 Mr Sd	12+
Hp-2	X_+X_+	1900-12 $Y_{Sp}Y_{Ty}{}^2$	1922	none			35 Sp	52 Ty
Jp	$X_{Dr}X_{Dr}$	1900-13 $Y_+Y_{Yo\delta p}$	1964	none			29 Dr	43 Vo Dr Sp
Cp	X_+X_{Sp}	1900-14 $Y_{Dr}Y_{Br}$	2005	none			10 Br	13 Sp Br
Jp	$X_{Dr}X_{Dr}$	1901-11 $Y_{Mr}Y_{Ir}$	1970	none			45 Dr Ir	51 Dr Mr
Jp	$X_{Dr}X_{Dr}$	1901-12 $X_{Ay\delta r}Y_+$	1921	21 Dr Ay Sr			20 Dr	
Further evidence that *Ay Sr* of 1901-12 is X-linked.								
1921-1	$X_{Dr}X_{Ay\delta r}$	1970-12 $X_{Dr}Y_{Mr}$	2010	12 Dr	15 Dr Ay Sr	1+	14 Dr Mr	10 Mr Ay Sr
Hp-2	X_+X_+	1901-12 $X_{Ay\delta r}Y_+$	1977	20 Ay Sr			21+	

1 Ay Sr

1 Ir Mr Dr

12+ 14 Dr Sp

Sd — Spotted-dorsal: irregular spots of macromelanophores in the dorsal fin.

f-Sd — Forward spotted-dorsal: irregular spots of macromelanophores in the dorsal fin accompanied by heavy spotting on flank anterior to dorsal fin.

Sr — Stripe-sided: macromelanophores arranged in horizontal rows along the flanks.

Sp — Spot-sided: small irregular spots of macromelanophores along the flank. Genetic tests, to be published elsewhere, have shown that two *Sp* alleles, designnated as Sp^7 and Sp^8, give rise to somewhat different spotted patterns. The differences are heritable. As far as is known the Sp^7 and Sp^8 alleles are also different from the *Sp* alleles of all other platyfish populations (Pl. I and Pl. II). Sp^7 is present in pedigrees 1937, 1968 (Table 2) and 1930, 1931 (Table 3). Sp^8 is present in pedigrees 1922, 1964 (Table 1) and 1927 (Table 2). In these experiments the differences between the spotted patterns were not important and the particular *Sp* alleles have usually not been identified.

Red and yellow body and fin patterns:

The red and yellow patterns of *Xiphophorus maculatus* are formed by xanthophores and xantho-erythrophores. Goodrich, Hill, and Arrick (1941) and Öktay (1964) have identified the red pigments as pterin compounds while the nature of the yellow pigment is still in doubt. According to preliminary results, certain xanthophores contain colorless pterins and carotenoid pigments (Öktay 1964).

CPo — Caudal peduncle orange: Three factors present in the population give rise to somewhat similar patterns. All of them exhibit incomplete penetrance and vary in their expression. In general the coloration is stronger in males than in females. The factor present in peds. 1937 (Table 2) and 1904 is concerned with background coloration; the factors in peds. 1909, 1929, 1972 (Table 3), 1914, 1915, and 1920 (Table 4) may give rise to bright golden-yellow or red pigmentation. Genetic tests have shown the patterns of peds. 1904, 1909, and 1920 to be controlled by different factors (Kallman unpublished). In this study, however, the patterns were treated as if they were caused by the same allele.

Ar — Anal red: anal fin or gonopodium assumes a red or orange coloration. The *Ar* patterns of the Belize and Jamapa populations are caused by different alleles. They are both present in ped. 1936 and 2050 (see below). The differences between the two alleles will be presented in a forthcoming paper.

Mr — Mouth red: in males lower jaw and gular region red; pattern poorly expressed in females.

Ay — Anal yellow: area above anal fin covered by yellow pigment cells; in some fish other parts of the body are also affected.

Br — Body red: this pattern is especially strongly developed along the flank behind the operculum. The tissue behind the two ventral most scale rows is not pigmented.

Vo — Ventral orange: orange coloration along the ventral most scale rows from area of the heart to insertion of anal fin. The pattern bears a superficial resemblance to Gordon's (1951a) pattern "ruby throat," *Rt*, but *Vo* lacks the characteristic bands of red pigments running dorsally. *Vo* is not expressed in Belize females.

Dr — Dorsal red: dorsal fin orange red.

Ty — Tail yellow: caudal fin a rich golden yellow.

Iris pattern:

Iy — Iris yellow and *Ir* — iris red: Most platyfish of natural populations have silvery grey irises with only traces of yellow pigment. Fish have been collected with irises that were either bright red or yellow. Genetic tests (Kallman unpublished) have shown that this pigmentation is controlled by two sex-linked factors. *Iy* gives rise to yellow pigment in females and young males, but in older males red pigment cells may also develop. *Ir* causes the appearance of red pigment cells in the irises of males and females. Both patterns are more strongly developed in males. The phenotypes of the most strongly pigmented *Iy* fish and the most weakly pigmented *Ir* fish overlap. The *Ir* factor was present in 1901-11 (Table 1 and Table 4) and 1899-9 (Table 2): *Iy* was found in 1899-11 (Table 1), 1899-1 and -8 (Table 2), 1900-2 and -7 (Table 3) and in ped. 1918 (Table 4). The male parent of ped. 1935 and ped. 1968 (Table 1 and Table 2) was heterozygous for both *Iy* and *Ir*.

The factors controlling iris coloration, red or yellow body and fin pigmentation and macro-

melanophore patterns are controlled by at least three major loci. According to Gordon (1937) and MacIntyre (1961), the macromelanophore system may represent a pseudoallelic series, since crossing over within the macromelanophore locus has been observed. An exceptional recombinant in which the *Sp* and *Sr* factors of the Jp 163 B stock may have become linked on the *Y* chromosome has been obtained in ped. 1981 (Table 2). Evidence which will be presented in greater detail elsewhere has shown that *Dr* of Jamapa and *Mr* of Belize are not allelic. Of all known pigment genes, the iris locus is located closest to the sex differential segment.

The origin and the sex chromosome constitution of the reference stocks with the exception of the *Up* strains has been described elsewhere (Kallman 1965). Strain *Up*-2 (*WY-YY*) has been derived from fish collected at Sebol, near the source of the Rio de la Pasion. Fish of pedigrees 1334, 1340, and 1375 are the progenitors of the stock (Kallman 1965, Table 17). Strain *Up*-1 (*XX-XY*) has been derived from fish of pedigree 1424 (Kallman 1965, Table 17). The following sex-linked patterns of the reference stocks were used as marker genes of their sex chromosomes:

	♀♀	♂♂
Hp-2	X_+X_+	X_+Y_{Sd}
Cp	$X_{Sp}X_+$	
Gp		$X_{Sp}Y_{Sd}$
Up-1	$X_+X_{f\text{-}Sd}$	
Up-2		$Y_{Sr}Y_{Iy}$
Jp 163 A	$X_{Dr}X_{Dr}$	$X_{Dr}Y_{Sr}$
Jp 163 B	$X_{Sp}X_{Sp}$	$X_{Sp}Y_{Sr}$ or $X_{Sp}Y_{ArSr}$

The method for maintaining fish, recording data and assigning pedigree numbers has been explained previously (Kallman 1965).

Results

Sex chromosomes of males: A total of eight males collected from the Sibun and Belize River drainages were crossed to *XX* females of the reference stocks. The *YY* genotype of seven males was clearly indicated by their all male offspring (Table 1). Such broods are characteristic of *XX* ♀♀ x *YY* ♂♂ crosses (Kallman 1965, Table 23). When five of the males were mated to *WY* females from the Belize River and Sibun River, young of both sexes were obtained in frequencies that did not significantly deviate from the theoretical 1:1 ratio (ped. 1927, 1968, Table 2; 1931, 1934, Table 3; 1920, Table 4). However, an eighth male, 1901-12, was *XY:* he gave rise to males and females in equal numbers; *Ay* and *Sr* were inherited in females only (ped. 1921, Table 1). Since the other seven males collected were *YY*, more evidence for the *XY* genotype of 1901-12 was desired. Therefore, this male was tested with an *XX* female of a second strain (Hp-2). Again the results are only consistent with the assumption that 1901-12 was *XY* with *Ay* and *Sr* linked on the *X* chromosome (ped. 1977, Table 1). Additional data was obtained from a cross involving a female descendant of male 1901-12 (ped. 2010, Table 1). The single wild type female of ped. 2010 is presumably due to nonexpression of *Dr* which was poorly developed in many females. The exceptional *AySr* male of ped. 2010 is a crossover which establishes that *Dr* of Jamapa is not allelic to *Mr* of Belize (Kallman, unpublished).

Sex chromosomes of females: The sex chromosomes of 18 females were identified by crossing them with *XY* males of the reference stocks (1899-1, -5, -7, -8, -9, Table 2; 1900-1, -3, -5, -6, -8, -10, -23, -24, -25, Table 3; 1901-1, -2, -3, -4, Table 4). The *X*- and *Y*-linked pigment patterns of the male parent were inherited by offspring of both sexes, thereby establishing the *WY* genotype of the 18 females. Moreover, with few exceptions, the maternal pigment patterns were inherited by males only. The young of eight other females (ped. 1916, 1927, 1968, Table 2; ped. 1905, 1909, 1934, 1931, Table 3; ped. 1920, Table 4) were either sired by *YY* males in the laboratory or by unknown males which had inseminated the females before they were collected. The evidence that the sex chromosome constitution of these eight females was *WY* is presented in Table 5: when a male offspring of each of the eight pedigrees was crossed to *XX* females, broods consisting only of males resulted. In ped. 1913 and 1937 (Table 2) the patterns of the P_1 female were inherited by males only but no further crosses were made with these fish. Females 1899-2 and -6, therefore, possessed a *W* chromosome and either an *X* or *Y* on which the pigment genes were located. The genotype of female 1900-22 was *WY*, although this is not readily apparent from the inheritance of pigment patterns and sex ratio of ped. 1952 (Table 3) in which an exceptional class of offspring was present (the *Sd* males). This cross is further analyzed below (Table 6).

The genotype of female 1900-21 was *XX*. This is indicated by the 1:1 sex ratio and the inheritance of *Sd* by all males but none of the females of ped. 1923 (Table 3). Two crosses provide further evidence for the *XX* genotype of 1900-21. A *Sd* male of ped. 1923 without the *Ar* pattern was crossed with *Jp 163 B*. In their progeny (ped. 2033) *Sd* was inherited only by males. Therefore, the unmarked sex chromosome of female 1900-21 was X_+.

Jp $X_{Sp}X_{Sp}$ x 1923-11 X_+Y_{Sd}
Ped. 2033: 23 *Sp*♀♀; 13 *SpSd*♂♂

TABLE 2. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF TEN FEMALES (ped. 1899 Collected from the Sibun River, British Honduras (Freetown).

Pedigree and genotype of parents				*Pedigree of offspring*			
♀♀		♂♂			♀♀		
1899-3	W_+Y_+†	unknown		1916	8 Ay*	7 +	
1899-5	W_+Y_+	Gp	$X_{Sp}Y_{Sd}$	1933	23 Sp	18 Sd	1
1899-7	W_+Y_{Ay}	Hp-2	X_+Y_{Sd}	1932	16 Sd	19 +	
1899-2	$W_+?_{Ay}$	unknown		1913	8 Dr*	19 +	
1899-9	W_+Y_{Ir}	Jp	$X_{Sp}Y_{Sr}$	1981	24 Sp	14 Sr	
1899-4	W_+Y_{Sd}	1900-13‡	Y_+Y_{VoSp}	1927	17 Sp[2]	16 +	
1899-6	$W_+?_{AySp}$	unknown		1937	11 CPo*	11 Sd*	17
1899-10	W_+Y_{AySp}	1899-12‡	$Y_{Iy}Y_{Ir}$	1968	23 Ir	11 Iy	
1899-1	W_+Y_{IyAy}	unknown		1906a	7 Mr Sd*	9 +	
1899-1		Hp-2	X_+Y_{Sd}	1906b	12 Sd	9 +	
1899-8	W_+Y_{IyAy}	Jp	$X_{Sp}Y_{Sr}$	1928	17 Sp	11 Sr	

†The sex chromosomes listed for the ten females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1899-12 and 1900-13 are identified in Table 1.
*Some patterns inherited from unknown male parent.
[1] An exceptional offspring with a new macromelanophore pattern linked to *Ar* on Y chromosome (Kallman unp
[2] *Vo* is not expressed in females; 7 males were sacrificed at the age of 5 months before the pattern was apparent.
[3] Some fish also exhibit some red coloration in iris.

TABLE 3. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIFTEEN FEMALES (ped. 19 Collected from the Belize River, British Honduras (Bermudian Landing)

Pedigree and genotype of parents				*Pedigree of offspring*			
♀♀		♂♂			♀♀		
1900-1	W_+Y_{CPo}	unknown		1904a	14 +	9 N*	5 Sd*
1900-1		Jp	$X_{Sp}Y_{Sr}$	1904b	11 Sp	9 Sr	
1900-5	W_+Y_{Sr}	unknown		1911a	13 +	1 Sd Mr*	
1900-5		Hp-2	X_+Y_{Sd}	1911b	21 +	18 Sd	
1900-22	W_+Y_N	Gp	$X_{Sp}Y_{Sd}$	1952	24 Sp	3 Sd	
1900-24	W_+Y_N	Hp-2	X_+Y_{Sd}	1953	20 Sp	15 Sd	
1900-8	W_+Y_{Ay}	unknown		1917a	9 +	10 Mr Sd*	
1900-8		Gp	$X_{Sp}Y_{Sd}$	1917b	1 Sp	10 Sd	
1900-2	W_+Y_{Iy}	unknown		1905	29 +	1 Ir*	1 Mr*
1900-10	W_+Y_{Ar}	Jp	$X_{Dr}Y_{SrAr}$	1936	18 Sp	20 Sr Ar[1]	
1900-9	W_+Y_{AySp}	1900-14‡	$Y_{Dr}Y_{Br}$	1931	7 +	18 Br	
1900-23	W_+Y_{AySp}	Jp	$X_{Dr}Y_{Sr}$	1930	22 Sr	17 Dr	13 Dr
1900-6	W_+Y_{CPoSr}	Hp-2	X_+Y_{Sd}	1929	34 +	35 Sd	
1900-4	W_+Y_{CPoSr}	unknown		1909	53 +		
1900-25	W_+Y_{CPoN}	Jp	$X_{Dr}Y_{Sr}$	1972	10 Dr	14 Sr	
1900-3	W_+Y_{BrSd}	unknown		1908a	15 +	11 Dr*	
1900-3		Jp	$X_{Sp}Y_{Sr}$	1908b	19 Sp	18 Sr	
1900-7	W_+Y_{IyAy}	1900-11‡	Y_+Y_{MrSd}	1934	9 +	10 Mr Sd	
1900-21	X_+X_{Ar}	Hp-2	X_+Y_{Sd}	1923	19 +	10 Ar	

†The sex chromosomes listed for the 15 females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1900-11 and 1900-14 are identified in Table 1.
*Some pattern inherited from unknown male parent.
[1] Anal red, *Ar*, pattern of 1900-10 and of the 35 male offspring is different from that present in the 20 *Sr* females.
[2] One male of each class developed some orange coloration in the iris.

Phenotype of offspring		
	♂♂	
7 Ay*	10 +	
9 Sp	30 Sd	
1 Ay Sd	15 Ay	
0 Ay Dr*	16 Ay	1 Iy Ay*
6 Ir *Sp*	11 Ir Sr	1 Ir M[1]
3 Vo Sp Sd	28 Sd	7 Sp Sd[2]
1 Ay Sp CPo*	5 Ay Sp Sd*	19 Ay Sp
1 Iy Ay Sp	28 Ir Ay Sp	
5 Iy Ay Mr Sd*	14 Iy Ay[3]	1 +
2 Iy Ay Sd	12 Iy Ay	
9 Iy Ay Sp[3]	19 Iy Ay Sr[3]	

henotype of offspring		
	♂♂	
6 CPo	2 CPo N*	
7 CPo Sp	7 CPo Sr	
1 Sr		
3 Sr	12 Sd Sr	
2 N Sd	9 Sp N	13 Sd
0 N Sd	9 N	
0 Ay Mr Sd*	9 Ay	
5 Ay Sd	5 Ay Sp	
6 Iy	14 Ir*	2 Mr* 5 +
6 Sp Ar[1]	19 Sr Ar[1]	
5 Ay Sp Dr	12 Br Ay Sp	
4 Ay Sp Sr	13 Dr Ay Sp	
5 Sd CPo Sr	25 CPo Sr	
3 CPo Sr		
2 CPo N Sr	20 CPo N Dr	
3 Br Sd	12 Br Sd Dr*	
3 Br Sd Sr	10 Br Sd Sp	
9 Iy Ay[2]	7 Iy Ay Mr Sd[2]	
7 Ar Sd	20 Sd	

A female of ped. 1923 with *Ar* was crossed to a *Jp* male. All female offspring but none of the male offspring (ped. 2050) inherited *Sp*, while *Sr* was inherited by males only. Therefore the genotype of 1923-1 must have been *XX*, one *X* derived from *Hp-2*, and the other, marked by *Ar*, from 1900-21.

$$1923\text{-}1\ X_{+}X_{Ar} \quad x \quad Jp\ X_{Sp}Y_{ArSr}$$

Ped. 2050: 18 *Sp*♀♀; 13 *SpAr*♀♀; 33 *ArSr*♂♂

In ped. 2050 the *Ar* progeny fell into two non-overlapping classes. The 13 *Ar* females and 19 of the *Ar* males exhibited a red pattern quite unlike that present in *Jp* fish with *Ar* or in Jp x Belize hybrids that inherited the *Ar* factor of Jamapa.

The unknown male which had already fertilized female 1900-2 (Table 3) at the time of capture, must have possessed the *XY* genotype. A wild-type female offspring (1905-2) was crossed to 1904-11, a $Y_{CPo}Y_{N}$ male. They produced young of four classes (ped. 2124): 7 N♀♀, 8 + ♀♀, 3 N♂♂ and 2 *CPo*♂♂. The eight wild-type females presumably carry the *CPo* allele which is poorly expressed in many females. When a *N* male (2124-12) was bred with a Jp $X_{Dr}X_{Dr}$ female, all nine *Dr* offspring were females, all 13 *Dr N* were males (ped. 2245). Similarly a male with the *CPo* pattern (2124-11) sired 15 *Dr* females and 30 *Dr CPo* males (ped. 2234), when mated with a Jp female. These results indicate that *CPo* and *N* are *Y*-linked (already confirmed for *CPo* by ped. 1904 b, Table 3). The other sex chromosome of males 2124-11 and -12 must have been an unmarked X, traceable to the unknown male that fertilized 1900-2. The genotype of the wild-type female, 1905-2, was *WX*.

Among the pedigrees listed in Tables 2, 3, and 4 are several exceptions that require further explanation. If the genotype of female 1900-2 were $W_{+}Y_{Iy}$, only males of pedigree 1905 should have inherited the iris pattern (Table 3). However, there were seven female young with iris coloration and seven male offspring without any. Pedigree 2084 (Table 6) demonstrates that *Iy* is Y-linked in females of ped. 1905. The females must have inherited the iris pattern from one of the unknown males that had inseminated female 1900-2 before she was collected. One of the males (1905-11, Table 5) with wild-type iris coloration was tested. He sired 32 *Mr* males and 29 *Iy* males: the *Iy* allele remained unexpressed in this male parent. However, it cannot be assumed that the other exceptional males (ped. 1905) were also due to nonexpression. The two males of peds. 1906a and 1907b (Table

TABLE 2. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF TEN FEMALES (ped. 189
Collected from the Sibun River, British Honduras (Freetown).

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*		
♀♀		♂♂			♀♀		♂♂
1899-3	W_+Y_+†	unknown		1916	8 Ay*	7 +	7 Ay* 10 +
1899-5	W_+Y_+	Gp	$X_{Sp}Y_{Sd}$	1933	23 Sp	18 Sd	19 Sp 30 Sd
1899-7	W_+Y_{Ay}	Hp-2	X_+Y_{Sd}	1932	16 Sd	19 +	11 Ay Sd 15 Ay
1899-2	$W_+?_{Ay}$	unknown		1913	8 Dr*	19 +	10 Ay Dr* 16 Ay 1 Iy Ay*
1899-9	W_+Y_{Ir}	Jp	$X_{Sp}Y_{Sr}$	1981	24 Sp	14 Sr	16 Ir Sp 11 Ir Sr 1 Ir M[1]
1899-4	W_+Y_{Sd}	1900-13‡	Y_+Y_{voSp}	1927	17 Sp[2]	16 +	13 Vo Sp Sd 28 Sd 7 Sp Sd[2]
1899-6	$W_+?_{AySp}$	unknown		1937	11 CPo*	11 Sd*	11 Ay Sp CPo° 5 Ay Sp Sd* 19 Ay Sp
1899-10	W_+Y_{AySp}	1899-12‡	$Y_{Iy}Y_{Ir}$	1968	23 Ir	11 Iy	11 Iy Ay Sp 28 Ir Ay Sp
1899-1	W_+Y_{IyAy}	unknown		1906a	7 Mr Sd*	9 +	5 Iy Ay Mr Sd* 14 Iy Ay[3] 1 +
1899-1		Hp-2	X_+Y_{Sd}	1906b	12 Sd	9 +	12 Iy Ay Sd 12 Iy Ay
1899-8	W_+Y_{IyAy}	Jp	$X_{Sp}Y_{Sr}$	1928	17 Sp	11 Sr	19 Iy Ay Sp[3] 19 Iy Ay Sr[3]

†The sex chromosomes listed for the ten females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1899-12 and 1900-13 are identified in Table 1.
*Some patterns inherited from unknown male parent.
[1] An exceptional offspring with a new macromelanophore pattern linked to *Ar* on Y chromosome (Kallman un
[2] *Vo* is not expressed in females; 7 males were sacrificed at the age of 5 months before the pattern was apparent.
[3] Some fish also exhibit some red coloration in iris.

TABLE 3. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIFTEEN FEMALES (ped. 1
Collected from the Belize River, British Honduras (Bermudian Landing)

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*			
♀♀		♂♂			♀♀			♂♂
1900-1	W_+Y_{CPo}	unknown		1904a	14 +	9 N*	5 Sd*	2 CPo N*
1900-1		Jp	$X_{Sp}Y_{Sr}$	1904b	11 Sp	9 Sr		7 CPo Sp
1900-5	W_+Y_{Sr}	unknown		1911a	13 +	1 Sd Mr*		11 Sr
1900-5		Hp-2	X_+Y_{Sd}	1911b	21 +	18 Sd		13 Sr 12 Sd Sr
1900-22	W_+Y_N	Gp	$X_{Sp}Y_{Sd}$	1952	24 Sp	3 Sd		12 N Sd 9 Sp N 13 Sd
1900-24	W_+Y_N	Hp-2	X_+Y_{Sd}	1953	20 Sp	15 Sd		10 N Sd 9 N
1900-8	W_+Y_{Ay}	unknown		1917a	9 +	10 Mr Sd*		10 Ay Mr Sd* 9 Ay
1900-8		Gp	$X_{Sp}Y_{Sd}$	1917b	1 Sp	10 Sd		5 Ay Sd 5 Ay Sp
1900-2	W_+Y_{Iy}	unknown		1905	29 +	1 Ir*	1 Mr*	16 Iy 14 Ir* 2 Mr* 5 +
1900-10	W_+Y_{Ar}	Jp	$X_{Dr}Y_{SrAr}$	1936	18 Sp	20 Sr Ar[1]		16 Sp Ar[1] 19 Sr Ar[1]
1900-9	W_+Y_{AySp}	1900-14‡	$Y_{Dr}Y_{Sr}$	1931	7 +	18 Br		5 Ay Sp Dr 12 Br Ay Sp
1900-23	W_+Y_{AySp}	Jp	$X_{Dr}Y_{Sr}$	1930	22 Sr	17 Dr	13 Dr	14 Ay Sp Sr 13 Dr Ay Sp
1900-6	W_+Y_{CPoSr}	Hp-2	X_+Y_{Sd}	1929	34 +	35 Sd		25 Sd CPo Sr 25 CPo Sr
1900-4	W_+Y_{CPoSr}	unknown		1909	53 +			53 CPo Sr
1900-25	W_+Y_{CPoN}	Jp	$X_{Dr}Y_{Sr}$	1972	10 Dr	14 Sr		12 CPo N Sr 20 CPo N Dr
1900-3	W_+Y_{BrSd}	unknown		1908a	15 +	11 Dr*		13 Br Sd 12 Br Sd Dr*
1900-3		Jp	$X_{Sp}Y_{Sr}$	1908b	19 Sp	18 Sr		13 Br Sd Sr 10 Br Sd Sp
1900-7	W_+Y_{IyAy}	1900-11‡	Y_+Y_{MrSd}	1934	9 +	10 Mr Sd		9 Iy Ay[2] 7 Iy Ay Mr Sd[2]
1900-21	X_+X_{Ar}	Hp-2	X_+Y_{Sd}	1923	19 +	10 Ar		7 Ar Sd 20 Sd

†The sex chromosomes listed for the 15 females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1900-11 and 1900-14 are identified in Table 1.
*Some pattern inherited from unknown male parent.
[1] Anal red, *Ar*, pattern of 1900-10 and of the 35 male offspring is different from that present in the 20 *Sr* female
[2] One male of each class developed some orange coloration in the iris.

A female of ped. 1923 with *Ar* was crossed to a *Jp* male. All female offspring but none of the male offspring (ped. 2050) inherited *Sp*, while *Sr* was inherited by males only. Therefore the genotype of 1923-1 must have been *XX*, one *X* derived from *Hp-2*, and the other, marked by *Ar*, from 1900-21.

$$1923\text{-}1\ X_+X_{Ar} \quad x \quad Jp\ X_{Sp}Y_{ArSr}$$

Ped. 2050: 18 *Sp*♀♀; 13 *SpAr*♀♀; 33 *ArSr*♂♂

In ped. 2050 the *Ar* progeny fell into two non-overlapping classes. The 13 *Ar* females and 19 of the *Ar* males exhibited a red pattern quite unlike that present in *Jp* fish with *Ar* or in Jp x Belize hybrids that inherited the *Ar* factor of Jamapa.

The unknown male which had already fertilized female 1900-2 (Table 3) at the time of capture, must have possessed the *XY* genotype. A wild-type female offspring (1905-2) was crossed to 1904-11, a $Y_{CPo}Y_N$ male. They produced young of four classes (ped. 2124): 7 N♀♀, 8+ ♀♀, 3 N ♂♂ and 2 *CPo* ♂♂. The eight wild-type females presumably carry the *CPo* allele which is poorly expressed in many females. When a *N* male (2124-12) was bred with a Jp $X_{Dr}X_{Dr}$ female, all nine *Dr* offspring were females, all 13 *Dr N* were males (ped. 2245). Similarly a male with the *CPo* pattern (2124-11) sired 15 *Dr* females and 30 *Dr CPo* males (ped. 2234), when mated with a Jp female. These results indicate that *CPo* and *N* are *Y*-linked (already confirmed for *CPo* by ped. 1904 b, Table 3). The other sex chromosome of males 2124-11 and -12 must have been an unmarked X, traceable to the unknown male that fertilized 1900-2. The genotype of the wild-type female, 1905-2, was *WX*.

Among the pedigrees listed in Tables 2, 3, and 4 are several exceptions that require further explanation. If the genotype of female 1900-2 were W_+Y_{Iy}, only males of pedigree 1905 should have inherited the iris pattern (Table 3). However, there were seven female young with iris coloration and seven male offspring without any. Pedigree 2084 (Table 6) demonstrates that *Iy* is Y-linked in females of ped. 1905. The females must have inherited the iris pattern from one of the unknown males that had inseminated female 1900-2 before she was collected. One of the males (1905-11, Table 5) with wild-type iris coloration was tested. He sired 32 *Mr* males and 29 *Iy* males: the *Iy* allele remained unexpressed in this male parent. However, it cannot be assumed that the other exceptional males (ped. 1905) were also due to nonexpression. The two males of peds. 1906a and 1907b (Table

2 and Table 4) lacking maternal pigment patterns were genetic sex reversals of the *WY* genotype (Table 6). This is evinced by the inheritance of *CPo* and *Sr* in all male and some of the female offspring of ped. 2146 and by the appearance of *Sr* females (*WX*) and *SdSr* males (*XY*) in ped. 2069.

Most probably the *Sd* males of ped. 1952 (Table 3) were genetic sex reversals of the *WY* genotype. None were tested, because an earlier analysis of a similar situation had shown that *WY* males often give rise to many more sex reversals (Kallman 1968). Such results would not help to identify the sex chromosome constitution of female 1900-22. Instead, one fish each of the "normal' classes was tested (Table 6). Ped. 2065 demonstrates that the *N* gene of female 1900-22 was *Y*-linked; ped. 2071 and 2090 show that her unmarked sex chromosome was a W. The eleven *Sr* and two *Iy* males of the last two pedigrees are also sex reversals. Thus fish of ped. 1952 were of the following genotypes: *Sp* ♀♀ – WX; *Sd* ♂♂ and ♀♀ – WY, *SpN* ♂♂ – XY; *SdN* ♂♂ – YY.

The occurrence of males with the exceptional genotype WY is not new for *X. maculatus* and one special case was analyzed recently (Kallman 1968). As in the previous study WY males arose not only among F_1 hybrids between two stocks, but were also found among the progeny when normal female sibs of sex reversals were mated to males of different, totally unrelated stocks (in these crosses to *Jp* and *Up,* peds. 2071 and 2090). Since the WY males of ped. 1952 (*Bp* ♀ x *Gp* ♂), ped. 2071 [(*Bp* ♀ x *Gp* ♂) ♀ x *Jp* ♂] and ped. 2090 [(*Bp* ♀ x *Gp* ♂) ♀ x *Up-2* ♂] obviously have different genotypes, relatively few factors, perhaps only one or two with incomplete penetrance, must cause WY fish to differentiate into functional males. In contrast to the descendants of *Np* x *Cp* crosses (Kallman 1968), no WX males were herein encountered.

Discussion

Breeding data involving fish collected in British Honduras have demonstrated that the majority of females are *WY* and males *YY*. However, one *XX* female and one *XY* male from the Belize River have been identified at Bermudian Landing and one *XY* male at Gaboural Creek. Therefore, the theory that the *X* chromosome is absent from *X. maculatus* populations inhabiting rivers in British Honduras can no longer be maintained. The *X* chromosome has been demonstrated throughout the range of *X. maculatus,* from the Rio Jamapa (Veracruz, Mexico) in the west to the Belize River in the east. There are a few locations where the *X* chromosome has not yet been found. No fish were examined from the Rio Tonala, Mexico. Only one female and two males were tested from the New River in British Honduras (Kallman 1965). Information is also lacking for several small populations of platyfish in the streams and creeks of the narrow coastal plain of British Honduras, south of the Sibun River, but these populations inhabit an area that comprises less than one per cent of the total range of this species.

The results of the crosses herein described suggest the hypothesis that the *W* chromosome of natural populations does not carry pigment factors. This is unexpected because crossing over of pigment genes from the *Y* to the *W* has been reported in domesticated stocks of platyfish (Bellamy and Queal, 1951; Fraser and Gordon 1929; Gordon 1937) and in laboratory stocks derived from wild populations (Kallman 1965). The 27 marked females collected in the Sibun and Belize Rivers (Tables 2, 3, and 4) possessed a total of 41 patterns controlled by sex-linked factors (4 ♀♀ – macromelanophores only; 11 ♀♀ – macromelanophores and red and yellow body patterns; 7 ♀♀ – red or yellow body patterns only; 2 ♀♀ – iris patterns only; 3 ♀♀ – red or yellow body and iris patterns). Of the 41 factors representing at least three loci, not one was *W*-linked. Since the females were obtained from three stations only, the sample may actually be smaller than it appears: several females of a collection could have been related and could have inherited their pigment genes from the same parent. Thus certain marked chromosomes would be represented more than once in the sample. This is probably true of the Sp^8 and *Vo* alleles. The combination Sp^8Vo was present in two *YY* males from Bermudian Landing. No other sexually mature fish with Sp^8 or *Vo* were collected[3]. However, even if each pattern or combination of patterns of each location is counted only once, the three collections combined are still comprised of 19 differently marked females with 28 pigment factors. The absence of *W*-linked patterns, therefore, does not appear to be a sampling error.

Certain red patterns develop poorly in females. Preliminary experiments have shown them to be under androgenic control. There exists the possibility that females might possess pigment factors on the *W* that would go undetected for many generations. It is difficult to surmise the possible function of a pigment factor that would be inherited strictly maternally but which could only be expressed in males. The possibility of

[3] An immature fish with Sp^8 was present in the Gabourel Creek collection. It was too young to have any *Vo* exhibited.

TABLE 4. INHERITANCE OF PIGMENT PATTERNS AMONG THE OFFSPRING OF FIVE FEMALES (ped. 1901)
Collected from the Belize River, British Honduras (Gabourel Creek)

Pedigree and genotype of parents				*Pedigree of offspring*	*Phenotype of offspring*					
♀♀		♂♂			♀♀			♂♂		
1901-1	W_+Y_{Ay}	unknown		1907a	4 N*	23 +		1 Ay N*	20 Ay	
1901-1		Hp-2	X_+Y_{Sd}	1907b	14 Sd	12 +		11 Sd Ay	5 Ay	1 Sd
1901-4	W_+Y_{Ay}	unknown		1918a	16 Dr°	19 +	5 Iy*	11 Ay Dr*	3 Ay Iy*	11 Ay
1901-4		Hp-2	X_+Y_{Sd}	1918b	4 Sd	5 +		2 Sd Ay	4 Ay	
1901-2	W_+Y_{CPoSd}	Jp	$X_{Sp}Y_{Sr}$	1914	21 Sr	33 Sp		25 Sp CPo Sd	30 Sr CPo Sd	
1901-3	W_+Y_{CPoSd}	Jp	$X_{Sp}Y_{Sr}$	1915	21 Sr	19 Sp		16 Sp CPo Sd	16 Sr CPo Sd	
1901-5	W_+Y_{CPoSr}	1901-11‡	$Y_{Mr}Y_{Ir}$	1920	19 Mr	9 Ir		21 CPo Sr Mr	15 CPo Sr Ir	

†The sex chromosomes listed for the five females are the only ones that will adequately explain the results.
‡Sex chromosomes of 1901-11 are identified in Table 1.
*Some patterns inherited from unknown male parent.

TABLE 5. IDENTIFICATION OF SEX CHROMOSOME CONSTITUTION OF EIGHT FEMALES FROM SIBUN RIVER AND BELIZE RIVER
(Crosses between XX females of reference stocks and male offspring of wild-caught females)

Pedigree and sex chromosomes of parents				*Pedigree of offspring*	*Phenotype of offspring*			
♀♀		♂♂			♀♀	♂♂		
Jp	$X_{Sp}X_{Sp}$	1916-11	$Y_{Ay}Y_+$	2119	none	20 Ay Sp	13 Sp	
Jp	$X_{Sp}X_{Sp}$	1927-12	$Y_{Sd}Y_{VoSp}$	2105	none	23 Vo Sp^{s}**	15 Sd Sp	
Up-1	X_+X_{f-Sd}	1968-11	$Y_{Ir}Y_{AySp}$	2053	none	5 Ay Sp	19 f-Sd Ay Sp	11 f-Sd Ir 10 Ir
Jp	$X_{Sp}X_{Sp}$	1905-11**	$Y_{Iy}Y_{Mr}$	2063	none	32 Mr	29 Iy	
Jp	$X_{Sp}X_{Sp}$	1909-12	Y_+Y_{CPoSr}	2106	none	26 Sp	20 Sp CPo Sr	
Jp	$X_{Sp}X_{Sp}$	1934-11	$Y_{MrSd}Y_{IyAy}$	2130	none	15 Iy Ay Sp	10 Sp Mr Sd	
Jp	$X_{Dr}X_{Dr}$	1931-12	$Y_{Br}Y_{AySp}$	2064	none	29 Dr Br	23 Ay Sp Dr	
Jp	$X_{Sp}X_{Sp}$	1920-11	$Y_{Mr}Y_{CPoSr}$	2057	none	27 Sp Mr	39 Sp CPo Sr	

*Sp^{s} masks Sp pattern of Jamapa stock.
**Phenotypically *Mr* when sacrificed at age of 20 months.

cryptic patterns does not apply to the macromelanophore alleles that are well expressed in both sexes, nor to iris coloration although it is generally more intense in males (Kallman unpublished). Four red or yellow body and fin patterns, *Br, Dr, Ar,* and *Ay,* develop quite strongly in both sexes, but the recognition of *Mr, Vo,* and certain *CPo* factors in females may be difficult or impossible[4]. *Mr* in females is not visible to the unaided eye but can be seen under higher magnification as an increased concentration of faintly orange pigment cells on the lower jaw. Since the poor expression of *Mr* in females was established soon after the beginning of the study of the Belize fish, all females and their offspring in Tables 2, 3, and 4 were examined for the pattern. Only two *Mr* females were discovered in pedigrees in which the pattern was not expected (ped. 1911, 1905, Table 3). *Vo* and occasionally *CPo* cannot be identified in females.

Breeding data published previously (Kallman 1965) are in accord with the hypothesis that the *W* chromosome of natural populations carries the wild-type allele at the macromelanophore locus. The patterns of 14 *WX* or *WY* females collected in the Belize River in 1949, New River, Rio Hondo (3 stations), Lake Peten, and Rio de la Pasion were controlled by *X*- or *Y*-linked factors.

It is also interesting to note that even in the domesticated stocks of the popular aquarium trade which have the *WY-YY* mechanism, *W*-linked pigment factors are usually absent. This is true of Gerschler's (1914) *X. maculatus* in Germany[5], Bellamy's platyfish at the University of Chicago and University of California (Bellamy 1922, 1928, 1933; Bellamy and Queal 1951), Gordon's fish at Cornell (Gordon 1927, 1937; Fraser and Gordon 1929)[6], Kosswig's and Breider's stocks in Germany (Kosswig 1928;

TABLE 6. EXPLANATION OF EXCEPTIONAL PROGENY FROM TABLES 2, 3, AND 4

Pedigree and genotype of parents			*Pedigree of offspring*	*Phenotype of offspring*			
♀♀		♂♂		♀♀		♂♂	
1905-1	$W+Y_{Ir}$	Jp $X_{Sp}Y_{Sr}$	2084	9 Sp	16 Sr	12 Ir Sp	11 Ir Sr
2049-6*	$W+Y_{CPoSr}$	1906a-13 $W+Y+$	2146	7 +	4 CPo Sr	6 CPo Sr	
Jp	$X_{Sr}X_{Sr}$	1907b-11 $W+Y_{Sd}$	2069	9 Sr		20 Sr Sd	
Jp	$X_{Sp}X_{Sp}$	1952-11 $Y_{Sd}Y_N$	2065	none		41 Sp Sd	32 Sp N
1952-1	$W+Y_{Sd}$	Jp $X_{Sp}Y_{Sr}$	2071	36 Sp	28 Sr	31 Sp Sd	18 Sd Sr 9 Sr
1952-2	$W+X_{Sp}$	Up-2 $Y_{Sr}Y_{Iy}$	2090	16 Sr	16 Iy	8 Sr Sp	16 Iy Sp 2 Iy 2 Sr

* From 1927 ♀ (Table 2) x 1909 ♂ (Table 3).

[4] No females with the *Ty* factor of the Belize population have been obtained; the expression of this pattern in Belize females remains unknown. A similar pattern, perhaps identical with *Ty* of Belize, occurs in the Up-1 stock of *X. maculatus* derived from fish collected in the headwaters of the Rio de la Pasion. In this stock a yellow to orange tail pattern is expressed in females.

[5] Gerschler described a cross between a *X. maculatus* female with "pulchra" pattern and a male of *X. hellerii*. In the F_1 generation "pulchra" was inherited by males only; actual numbers were unfortunately not given. Since F_1 *maculatus* x *hellerii* hybrids that have inherited the *W* chromosome of *maculatus* usually differentiate into females (see Kallman 1965, Table 26), it is most likely that pulchra was *Y*-linked.

[6] From Gordon's data (1951b) it cannot be determined whether the *W* chromosome of the Bh stock was marked by pigment genes.

TABLE 7. FREQUENCY OF MACROMELANOPHORE PATTERNS (M) IN COLLECTIONS OF PLATYFISH FROM THE BELIZE RIVER AND SIBUN RIVER (BRITISH HONDURAS)*

M Patterns	*G† 1949‡* ♀♀	♂♂	*G 1950* ♀♀	♂♂	*G 1951* ♀♀	♂♂	*G 1952* ♀♀	♂♂	*G 1966* ♀♀	♂♂	*B† 1966* ♀♀	♂♂	*F† 1966* ♀♀	♂♂
+	193	125	165	133	34	20	445	227	60	35	52	59	122	91
Sr		5	1				11	14	1	5	3	4	1	6
N	8	7	15	30	3	3	11	19	1	5	2	4		1
Sd	3	10	3	4	2	2	7	17	3	3	1	2	5	5
Sp[7]											2	6	5	3
Sp[8]							2	2		**		2		
SdN						1	1	2		2				
SdSr														
NSr		1						1						
Total fish	204	148	184	167	39	26	477	282	65	50	60	77	133	106
Total M fish	11	23	19	34	5	6	32	55	5	15	8	18	11	15
% M	5.4	15.5	10.3	20.4	12.8	24	6.7	19.5	7.6	30	13.3	23	8.3	14.2
Assumption I: Difference between males and females due to chance.														
Expect. M	20	14	28	25	7	3	54	32	11	9	11	15	14	11
χ^2	10.84		7.16		4.09		29		8.84		1.74		2.32	
P	0.001		<0.01		<0.05		<0.001		<0.01		>0.1		>0.1	
Assumption II: W chromosome is not marked.														
q***	0.08		0.11		0.13		0.11		0.16		0.12		0.08	
Expect. M ♀♀	16		18		5		50		10		7		11	
χ^2	0.26		0.066		0.001		7.26		2.92		0.16		0	
P	>0.1		0.8		0.98		>0.01		>0.05		>0.5		1	

*The figures for the Gabourel Creek collections from 1949-1952 are different from those listed by Gordon and Gordon (Table 15, 1957); however, both their data and ours show an excess of patterned males. Gordon and Gordon inexplicably mistook several males for females, confused *N* and *Sr* with *Sp*, and failed to recognize *Sd* patterns in several specimens. Difference in total number of fish is due to the fact that they rejected more fish as immatures.

†G = Gabourel Creek, B = Bermudian Landing, F = Freetown.

‡Year of collection.

**One immature fish with *Sp*[8] cannot be sexed.

***q = the frequency of marked Y chromosomes in the population.

Breider 1936)[7] and Kosswig's (1938) Fury strain. In an aquarium store in a small German town, Breider (1942) discovered a single female with a combination of patterns to suggest *W*-linkage and subsequent crosses proved this to be true. Since he was aware that the *W* chromosome is usually unmarked he suggested that the female most likely arose due to crossing over. The rarity of females with marked *W* chromosomes is also indicated by Gordon's report (1937) that inspection of two commercial aquarium establishments yielded only two females with two macromelanophore patterns each which upon breeding proved to be *W*- and *Y*-linked[8]. The absence of marked *W* chromosomes in commercial stocks appears quite significant, since breeders select for fish with the brightest colors or the most patterns.

The seven preserved collections of *X. maculatus* from the Belize River and Sibun River provide additional support for the hypothesis. Without exception, the proportion of males exhibiting macromelanophores in the samples was 2 to 3 times that of females (Table 7). In Table 7 it was first assumed that the number of patterned males and females differed due to chance; that the frequency of marked *W* and *Y* chromosomes was the same. Based upon this assumption, the deficiency of spotted females was highly significant for all but the two small 1966 collections from Bermudian Landing and Freetown, which nevertheless showed the same trend as the other samples.

However, if we assume that the *W* chromosome always carries the + allele, the frequency of patterned females should be less than that of males. From the number of males with macromelanophores the frequency of marked *Y* chromosomes in the population can be obtained (Table 7); this value should equal the percentage of females with patterns. A slight complication is introduced by (1) considering all females as *WY* and males as *YY* and thereby ignoring the few fish with *X* chromosomes, and by (2) possible nonexpression of macromelanophore alleles in young fish. In spite of these possible sources of error, the actual data of six of the seven collections fit the assumption rather well that the *W* carries the + allele at the macromelanophore locus. Only for the 1952 collection is there a deficiency of females, but here too the fit is much better for the second assumption.

Although the data of the preserved collections tell us nothing about the frequency of the red or yellow patterns, breeding experiments indicate that the *W* chromosome of natural populations lacks pigment factors at these loci as well. This is not true of the *X* chromosome. Two of the four *X* chromosomes recovered from the populations of the Belize River and Sibun River were marked, one by *Ar* and the other by *AySr*. Similarly, several *X* chromosomes of males and females collected at locations in Guatemala carried factors for macromelanophore (Kallman 1965) and a variety of red and yellow patterns (Kallman unpublished).

As Gordon and Gordon (1957) already noted, no consistent correlation can be established between the frequency of a population's patterned males versus females and its sex chromosome mechanism. In regions where the *X* chromosome seems to be more common than in the Belize River (or where the *W* chromosome may be absent altogether), the frequency of patterned females may equal that of males (Rio Hondo), may be significantly higher than that of males (Rio Papaloapan) or significantly lower (Rio Jamapa, Rio Coatzacoalcos). It must be admitted, however, that our knowledge of platyfish sex chromosome mechanisms of the Rio Jamapa and Rio Papaloapan (Gordon 1947) and the Rio Coatzacoalcos and Rio Hondo (Kallman 1965) is quited limited, therefore no precise statement concerning the relative frequency of *X* and *W* chromosomes or absence of the *W* is possible. According to data published previously (see Kallman 1965, Table 21) the *X* chromosome in these populations seems to be more common than in those of the Belize River or Sibun River.

The diverse populations of the major river systems cannot be equated. Significant differences exist in the frequency and occurrence of certain macromelanophore and tail patterns (Gordon and Gordon, 1957). The *N* pattern and two tail spot markings are absent from the Rio Jamapa. Other tail patterns are not found in populations of the Rio Usumacinta system and rivers of British Honduras. The frequency of fish with macromelanophore patterns ranges from 0.05 in the Rio Grijalva to 0.35 in the Rio Papaloapan. Recent experiments indicate that the *Sd* and *Sp* patterns of populations inhabiting different river systems are caused by different alleles (Kallman unpublished). The selective value of alleles that give rise to virtually identical patterns may not be the same in

[7] The single female with a marked *W* chromosome reported by Kosswig in 1936 was due to crossing over as explained in a later paper (Kosswig 1937).

[8] It is realized, of course, that *WY* females homozygous for a pattern or heterozygous with *W*-linkage cannot be told apart from heterozygous females with *Y*-linkage by mere inspection. However, in stocks in which the pigment gene is on the *W*, the pattern is exhibited by females only.

the various populations. The selective value may depend upon the frequency of other pigment factors in the gene pool and upon linkage with other pigment genes.

The sex-linked patterns of *X. maculatus* are controlled by at least three major loci in the following sequence: sex differential segment, locus for iris pattern, locus for red or yellow body pattern, locus for macromelanophore pattern (Kallman unpublished). Crossing over between the *W* and *Y* chromosomes or between the *X* and *Y* chromosomes involving the sex differential segment and macromelanophore locus occurs at a frequency of 0.2 to 0.3% (Kallman 1965; Bellamy and Queal 1951). Thus certain combinations of patterns with high selective values will be maintained and could spread through the population. In the experiments reported herein, only three crossovers were observed. So far no sex chromosome with three pigment factors has been identified from natural populations (Table 8), although one has been obtained through crossing over in the *Up* stock (Kallman unpublished).

Although nothing has been published as to what conditions contribute to the remarkable sex chromosome and pigmentary polymorphism of *X. maculatus* (Table 8), it is most likely that, in general, conspicuous pigmentation in males may be advantageous during courtship and agonistic behavior, but of disadvantage as far as predation is concerned. In females dull coloration may be favored. These factors seem to be involved in maintaining the pigmentary polymorphism of another poeciliid fish, *Poecilia reticulata.* Fisher (1930) and Sheppard (1953) pointed out that Winge's data (Winge, 1927) showed overwhelming *Y*-linkage for the incompletely sex-linked pigment patterns, but that in the absence of selection crossing-over should tend to equalize the percentage of pigment factors on the *X* and *Y* chromosomes. They suggested the deficiency of *X*-linked pigment factors indicated bright coloration is an advantage in males and a disadvantage in females. Haskins and Haskins (1950) and Haskins, Haskins, McLaughlin, and Hewitt (1961) have demonstrated in laboratory experiments that in mating competitions brightly-colored males enjoy an advantage over dull-colored ones. In predation experiments the situation was reversed. They were also able to show that *X*-linked patterns were relatively rare in natural populations exposed to fish predators. It must also be emphasized that in *P. reticulata* most of the sex linked pigment patterns are under androgenic control and are not expressed in females even when present in homozygous condition. Thus two mechanisms, one hormonal and the other chromosomal, tend to restrict patterns to males. Rosen and Tucker (1961) have noted that in poeciliid genera with short gonopodia males are usually more highly pigmented than females and display elaborate courtship behavior.

Certain similarities between *P. reticulata* and *X. maculatus* are apparent. Besides the absence of pigment factors from the strongly female-determining *W* chromosome in natural populations of *X. maculatus,* evidence for a selective advantage of bright coloration in males of this species comes from the observation that in the Belize population (and perhaps also in others) certain red patterns are much better developed in males while other patterns are under androgenic control and not expressed in females at all. No such sex difference has been noted in the development of macromelanophore patterns, except that Sp^8 males often exhibit a large black spot above the insertion of the gonopodium. The spot is not present in Sp^8 females (Pl. I, figs. 1 and 2).

Certainly the breeding systems in the eight major drainages are not the same. In populations with an *XX* ♀♀ x *XY* ♂♂ mechanism, the *Y* chromosome, aside from rare cases of crossing

Table 8. Pigment Factors or Combination of Pigment Factors* on the Sex Chromosomes of the Offspring of 38 *Xiphophorus maculatus* Collected from the Belize and Sibun Rivers, British Honduras

Freetown (ped. 1899)	*Bermudian Landing* (ped. 1900)	*Gabourel Creek* (ped. 1901)
	Sr	
Sd	Sd	
	N	N
Mr Sd	Mr Sd	
	Br Sd	CPo Sd
Ay Sp^7	Ay Sp^7	
	CPo Sr	CPo Sr
		Ay Sr (x)
	CPo N	
	Vo SP^8	
	Br	
Dr	Dr	Dr
Ay	Ay	Ay
	Ar	
	Ar (x)	
	Ty	
CPo	CPo	
	Mr	Mr
Iy	Iy	Iy
Ir	Ir	Ir
Iy Ay	Iy Ay	

*All pigment factors located on Y chromosome except those marked by (x).

over, essentially represents a vehicle for strict paternal transmission of characters, which ceases the moment a *W* chromosome arises. Factors on the *W* chromosome, irrespective of the frequency of the *X* in the gene pool, are strictly maternally inherited. In populations with a *XX-XY* mechanism, dull coloration in females and bright coloration in males theoretically could be achieved by restricting all color factors to the *Y* chromosome, or bringing the development of the patterns under hormonal control. Elimination of marked *X* chromosomes would be difficult, however, since they would be passed on to one half of the male offspring; such males would enjoy an advantage over those with an unmarked *X*, if bright coloration in males is at a premium. A system of absolute *Y*-linked pigment factors (holandric genes) would suffer from the disadvantage that crossing over would not be possible and that, consequently, the number of pattern combinations in a population would be fixed and limited. The evolution of a *W* chromosome might have been another means of insuring greater pigmentary polymorphism in males. A W_+ chromosome could spread readily in a population, because it would be inherited by none of the male offspring but by all female descendants of *WY* x *XY*, *WX* x *YY*, and *WY* x *YY* matings, and by two thirds of the female progeny of *WX* x *XY* crosses. W_+X and W_+Y females will be pigmented to a lesser degree than *XX* females and *XY* or *YY* males in populations in which pigment factors are restricted to *X* and *Y* chromosomes. On the other hand, marked *W* chromosomes that arose through crossing over would be exposed to negative selection in every generation and eventually lost. Perhaps this is one of the reasons why at present the W_+ chromosome is widespread and extremely common in some populations.

Summary

The teleost *Xiphophorus maculatus* (Poeciliidae) is polymorphic for sex chromosomes and sex-linked pigment patterns. Females of natural populations may be of the genotype *WY*, *WX*, or *XX* and males *XY* or *YY*. Fish of two populations from the Belize River and one from the Sibun River in British Honduras were tested for their sex chromosome constitution, because earlier but limited data had indicated that the *X* chromosome is absent from rivers in British Honduras (Gordon 1954).

Of six males examined from the Belize River, five possessed the *YY* and one the *XY* genotype. Of 20 females tested, 19 were *WY* and one *XX*. Breeding data showed that one of the *WY* females was fertilized by an unknown *XY* male before she was collected. No *X* chromosome was identified in the two males and ten females from the Sibun River.

Of the 29 females with a *W* chromosome all but two exhibited one or more sex-linked pigment patterns controlled by at least three loci: one concerned with iris coloration, a second with red or yellow body or fin pigmentation, and a third with macromelanophore patterns. The 27 females possessed a total of 41 pigment factors. Of these none was *W*-linked.

An analysis of the frequency of macromelanophore patterns in seven preserved collections from the Belize River and Sibun River showed that consistently the frequency of patterned males was two to three times that of females. This difference is in good agreement with the hypothesis that in natural populations the *W* chromosome does not carry pigment factors. This is not true for the *X* chromosome.

Since crossing over between the *W* and *Y* chromosome has been observed in the laboratory, it must also occur in natural populations. In the absence of selection crossing over should tend to equalize the frequency of marked *W* and *Y* chromosomes. A selective advantage is postulated for high coloration in males and a disadvantage in females. The significance of the *W* chromosome as a vehicle for strict maternal transmission of characters is discussed.

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EXPLANATION OF PLATES

PLATE I

FIG. 1. Female of ped. 1927 with Sp^8 pattern of Belize, 6 months. Macromelanophore spotting on flank extends as far anteriorly as the eye.

FIG. 2. Male of pedigree 2167 (descendant of ped. 1927), 9 months. Macromelanophore spotting on flank produced by Sp^8 extends over much of body. The largest macromelanophore spot is typically found above the insertion of the gonopodium. Black spotting in dorsal fin is caused by the *Sd* factor of Sibun river.

FIG. 3. Female 1899-10, 11 months after capture, with Sp^7 pattern. In contrast to Sp^8, spotting on flank is restricted to area below dorsal fin and caudal peduncle.

PLATE II

FIG. 1. Male of ped. 1968, 5 months. Sp^7 spotting is restricted to posterior part of body. In contrast to Sp^8 pattern only a single spot is found in front of level of dorsal fin and no large black spot develops above gonopodium.

FIG. 2. Female of ped. 1936, a Belize x Jamapa hybrid, with Sp^1 pattern from strain Jp 163 B, 7 months. Sp^1 factor in F_1 hybrids usually causes less than ten pigment spots. Expression becomes further reduced in backcross hybrids to Belize. Large black spot in front of dorsal fin is "shoulder spot" and markings at base of caudal fin are complete-crescent, *Cc*, and dot, *D*, tail patterns.